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Brief History and Advancements of Vaccination Against Avian Coccidiosis: A Review

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Abstract

Coccidiosis is a major protozoal disease that severely affects livestock and other animals, especially poultry. Seven species of *Eimeria* cause avian coccidiosis in poultry and evolve from the epithelial cells of intestine, readily induce illness and cause death to a varying extent. Prophylactic chemotherapy was a dominant choice for the control of coccidiosis but resistance to the drug was a major factor of therapy failure. Protective immunity was produced in chickens with any of *Eimeria* species as only species-specific immunity can be produced by recently used vaccines. Attenuation can be achieved by the serial passages in all seven *Eimeria* species. In chicken, the first attempt against coccidiosis caused the introduction of live oocysts, the basis of which led to the discovery of first live attenuated commercial vaccine, Paracox1. As the emerged recombinant vaccines were replaced as a first choice, there is still a dire need to do more work on new techniques like DNA vaccine formulation along with the role of dendritic cells to produce immunity and cross-protection against avian coccidiosis. This article describes step-by-step developments in the vaccination process from the last 70 years along with a brief discussion on novel techniques to induce immunity against coccidiosis.

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Introduction

In order to achieve full genetic potential, poultry should be raised and managed in excellent health status and in biosecure facilities to reduce the risk of transmissible diseases [1]. By 2050, the population of human beings will be almost 9 billion and the majority of protein requirements continue to deliver from the poultry sector for human consumption. Thus, the demand for poultry products such as meat and eggs is drastically increasing globally [1]. Maintaining gut integrity and intestinal health is an important issue in poultry throughout the production period. Coccidiosis is the major protozoal disease that disturbs gut integrity and intestinal health status. It is caused by the species of genus *Eimeria*, an apicomplexan protozoan parasite that typically infects cells of intestinal epithelium [2]. The reproductive cycle of *Eimeria* species has sexual as well as asexual phases so it is necessary to control the risk of "carryover" of oocysts from one generation of birds to another. Coccidiosis can cause severe intestinal damage, as it possesses explosive reproductive capacity within the intestines of hosts [3]. It attacks mucosal tissues, invades cells and causes lesions in the gut, which results in impaired digestion and poor nutrient absorption [4]. Control of coccidiosis is a major problem in the poultry sector. The preferred method to prevent coccidiosis for many decades is prophylactic chemotherapy, which includes specific anticoccidial drug usage. Resistance to a drug is a permanent feature that has to be dealt with accordingly. That's why no new anti-coccidial drug has been introduced into the market for many years [5]. This situation greatly urges the way for extensive vaccination of live attenuated vaccines. Vaccination is the most feasible method against *Eimeria* species as it develops strong, species-specific and protective immunity.

The work of poultry coccidia and coccidiosis was started in 1920s and at the beginning of 1930s. The scientists paved the basis for primary knowledge as in the form of speciation, pathogenicity, lifecycles, host specificity, introduction of protective immunity and prophylaxis by vaccination and chemotherapy [6]. About 50 years ago, a live vaccine was introduced in the USA of wild type. McDonald and Shirley worked a lot to derive attenuated lines of *Eimeria* during the late 1970s and 1980s through the process of serial passages in the chorioallantoic membrane, as done by Peter Long and colleagues. Paracox 1 was launched after a comprehensive study of precocious lines [7]. The journey continued and after six decades, researchers focused their attentions on DNA vaccine formulation to provoke cellular and immune responses against *Eimeria* [8]. This review paper has described the research work to gain knowledge about the control of avian coccidiosis through vaccination. The study will go on past, as it has well-described aspects of early researches that lead to vaccine development strategies along with current techniques and finally, the forthcoming by which a hypothesis will be made for development towards vaccines of novel recombinant origin which may substitute live vaccines.

History and advancements of vaccination

Previous works on *Eimeria* of poultry had demonstrated that vaccination against coccidiosis might be result-oriented on a large scale, which might be initiated by keeping in view the world scenario of this industry. The first point of interest was recognition of infection, infectious agent, its lifecycle along with knowledge of sexual and asexual phases of reproduction [9]. Secondly, the source of infection was important to understand, as poultry birds might get the infection by taking oocysts from infected feed [10]. The third step was to know about induction of immunity, which revealed that immunity can be produced by re-infecting the chickens with the same species of *Eimeria*. Immunity against coccidiosis may be induced by inoculation of very few oocysts, which posed no pathological signs [11]. Early researches also proved that no cross-immunity by vaccination can be achieved against different species of *Eimeria* [11]. Sulphaquinoxaline was known as a pioneering treatment for coccidiosis stated in an article published in 1948. This was the first paper to be published about the treatment of coccidiosis [4]. Here we will discuss different achievements while formulating vaccines against *Eimeria*.

Early work started in the era of 1960s and 1970s

In the 1960s, the synthesis of parasite-specific antibodies in the serum of birds was studied. It was the key factor related to immunity in the host against avian coccidiosis. At that time, there were some confusions related to the role of these antibodies as protective immunity agents. Later on,

information about the early response of immune system against coccidiosis was more clearly studied, which showed that serological response might be conferred by the three different immunoglobulins, IgM, IgA, and IgY. It was elucidated that the presence of an antibody was not necessary for conferring protective immunity to coccidiosis and it was confirmed through studies as perturbed by immunosuppression of B cells in chicken [12]. Samples were taken from serum, bile and intestinal washings to elicit an anti-*Eimeria* antibody response by standardization of enzyme-linked immunoassays. Through ELISA, antibodies were characterized and their kinetics were measured against different species of *Eimeria*. Additionally, the significance of IgA in coccidiosis was studied to know the existence of secretory immunoglobulins in the intestine of *Eimeria*-damaged chickens [13]. The idea was that immune cells are required to control oocyst production. A strategy was used in which comparisons of immune responses with only one infection or single infection with low doses of parasites, *i.e*., fifty oocysts per day for 1 to 2 weeks of life or trickle infections were analyzed. Results demonstrated that a trickle infection induced a little bit higher IgA level than a single infection. Due to the availability of very less information about cell-mediated immunity of the intestine, it was very hard to know the actual immunological reagents and established methodologies at that time. The Wattle swelling response of chickens showed delayed-type hypersensitivity, which was a rough idea of cell-mediated immunity at that time. Work on serial passages through chorioallantoic membranes of embryonated eggs by maintaining lines of *E. tenella* was also of considerable importance, which resulted in an increase in the production of oocysts within chorioallantoic membranes and lowering of virulence [13]. After serial passages, precocious lines characterized by the tremendous increment of production along with attenuation were developed [14, 15].

Analysis of genetic variation

The methods in practice for the identification of poultry coccidia until the mid-1970s were not much changed from those described upto1920s. The more pronounced methods in the form of electrophoretic mobility of enzymes came into existence in the 1970s, which offered high accuracy analysis of stocks of the *Eimeria* lining in laboratories [16]. As a result of this analysis, seven well-identified species of *Eimeria* causing disease in poultry were re-evaluated. This re-evaluation is still a topic of controversy but generally accepted today. It was very beneficial to clarify the identity of species in all coming years when modern live vaccines containing well-established lines or strains could be registered having important species of economic significance; including *E. mitis*, where re-establishment of the taxonomic status of the organism started in the mid-1970s [13]. At first, a single strain was used to provide immunity against coccidiosis but in the late 70s, antigenic diversity was used to formulate live vaccines [17].

Era of 1980s and 1990s

The 1980s is known as the molecular era of coccidiosis research as the molecular research against coccidiosis began to take off. With the introduction of different molecular studies, the modern coccidiologists entered into the field of the biology of *Eimeria* species to explore new ideas for chemotherapy, development of vaccines and new candidate antigens [18]. To identify molecules of the monoclonal antibody, different technologies were started in several laboratories. Different lifecycle stages of *Eimeria*, which are importantly involved in several sites, such as site-specificity, entry in the host cell, development within the body and evolution of protective immunity were studied. Administration and evaluation of candidate antigens for vaccine efficacy in chickens were examined [19]. Evaluation of species-specific immunity of *Eimeria* along with *Eimeria*-specific T-cell immunity was found [13]. Passive transfer of immunity against coccidiosis was demonstrated by Wallach and colleagues through the passive transfer of maternal IgG-mediated immunization [20]. In the 80s, precocious lines of all species emerged for the first time and the first live attenuated vaccines namely Paracox and Livacox were introduced in Europe [21].

Fiona Tomley started ground-breaking cellular and molecular studies in the late 1980s and early 1990s at the Institute for Animal Health (IAH), United Kingdom. The data and techniques developed in this study had a lot of potentials to deliver a new generation of vaccines. Biology of early lifecycle stages interrogated by Fiona Tomley and colleagues, including molecular organization structures, surface membranes and apical (secretory) organelles of merozoites and sporozoites [22, 23]. Studies on *E. tenella* were started in combination with sub-cellular fractionation

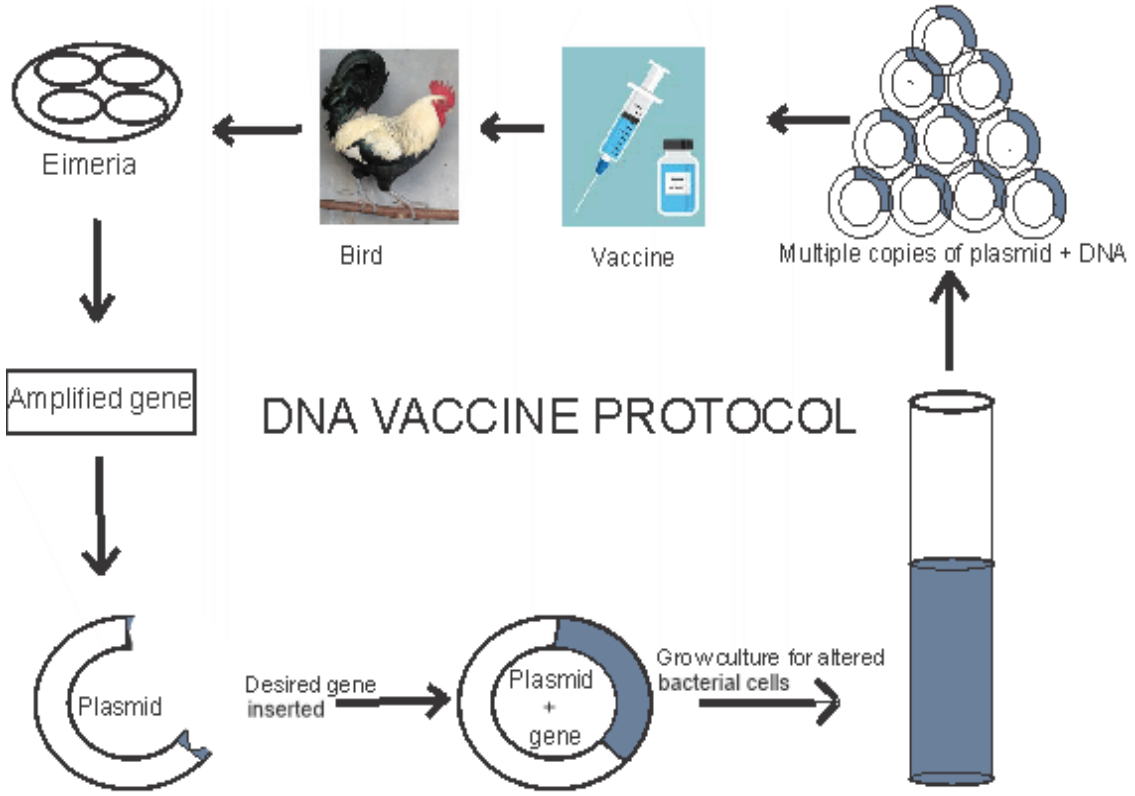

Fig. 1 Protocol for the synthesis of DNA vaccine against *Eimeria*.

techniques [22]. To understand the significance of proteins of *Eimeria* during the invasion, molecular cloning and sequencing helped a lot [23]. Fourteen chromosomes between 1 and 6 Mbp DNA sizes were found in *E. tenella* during studies on molecular karyotypes for *Eimeria* [24]. This work provided insight into genome sequencing studies. The host-parasite studies along with identification and characterization of chicken defense systems were observed. Effector molecules that take part in coccidial infection were also studied. Identification of new varieties of intestinal lymphocytes that responds to gut infection was done, which proved the notion that the host immune response is regulated by locally produced cytokines and chemokines [25]. It became clear that some most importantly involved mechanisms help to stop the further development of parasites within hosts, like acquired immunity towards coccidiosis. It was observed that within coccidia-immune hosts, further development of the parasite was inhibited when parasites entered the gut early after infection, for example, soon after infection of *E. tenella*, markedly increased cytotoxic T-lymphocytes expressing the CD^8 antigen were found in white Leghorn chickens [26]. Production of Interferon-g was also increased and it was later observed that interferon-g played a key role to inhibit intracellular development of *Eimeria* [27]. These all observations lead towards the introduction of different techniques like attenuation of live oocysts to develop vaccines. Live attenuated vaccines came into existence in various countries in the 1990s and 2000s, *e.g.*, ADVENT, Eimerivac, *Eimeria*vax, Gelcox, Inovocox, Nobilis, and CoxATM. Progression in the identification of species and strains along with electrophoretic polymorphism in DNA switched from the electrophoretic variation of isoenzymes in the 1990s, which gave the coccidiologists better access towards new techniques for vaccine formulation. These techniques could be used unequivocally and rapidly to identify small numbers of parasites and protozoa within the laboratories with the aid of standard molecular apparatus [13].

Era of 2000 onwards

The era of 2000 is also known as the era of biotechnological advancements. Several novel methods were used to create more efficient vaccines than ever before. Some of the prominent achievements are as follows.

Table 1 Role of different cytokines in DNA vaccines against *Eimeria* species.

Cytokine used	Eimeria species	Combined effects then gene alone	References
1. IL-1β	*Eimeria acervulina*	Better	[34]
2. IL-2	*Eimeria acervulina, Eimeria tenella*	Better	[31, 32, 34, 35, 36]
3. IL-8	*Eimeria acervulina*	Better	[34]
5. IL-10	*Eimeria maxima*	Better	[37]
6. IL-15	*Eimeria acervulina*	Better	[34]
7. IL-17	*Eimeria tenella*	Better	[38, 39]
8. IL-17A	*Eimeria tenella*	Better	[40]
9. IFN-α	*Eimeria acervulina,*	Better	[34]
10. IFN-γ	*Eimeria acervulina, Eimeria tenella*	Better	[31, 34, 41]

Molecular vaccines

Modern immunogenic techniques, like protein-based and DNA vaccines, were formulated using molecular biotechnology for coccidiosis in different Laboratories. Unlike protein-based vaccines, DNA vaccines comprise genes that encode immunogenic proteins. They are used along with promoters and enhancers. It was evident in a previous study that chickens who received a DNA vaccine encoding *E. acervulina* profiling protein showed a smaller number of oocysts in their fecal material [28].

DNA vaccines against coccidiosis

The DNA vaccine technology is a better approach towards prophylactic immunity and is referred to as a third-generation vaccine or immunological turning point [29]. The principle of this technology is very rationale. Immunogenic proteins are coded by genes of interest, then inserted into a plasmid which is an appropriate eukaryote, having the capability to replicate within bacteria. The plasmid is then purified and directly inoculated into the animal by various methods (Fig. 1). DNA vaccines are relatively cost-effective as they are easily generated, changed and marked. These vaccines can store with relative ease, as they do not need cold storage. Because of their chemical and structural characteristics, the stability of DNA vaccines is higher as compared to traditional vaccines that contain, highly virulent organisms as well as live antigens [30]. Several experiments have been performed regarding DNA vaccines encoding different *Eimeria* antigens in the last two decades. A trial was performed in which protective immunity posed by DNA of *E. acervulina* cSZ-2 and IL-2 was seen against *E. tenella* challenge [31]. Findings made it clear that cSZ-2 DNA immunization can induce host immune responses by decreasing body weight loss, intestinal lesions, and oocyst ratio. A DNA vaccine containing *E. tenella* TA4 and chicken IL-2 (chIL-2) was constructed and its efficacy was checked against *E. tenella* challenge [32]. Results revealed that antigen genes were expressed effectively *in vivo* with significant results. Similarly, a trial was performed, in which a gametocyte antigen GAM56 was used, to produce immunity against avian coccidiosis. The dose rate was also checked and results showed that GAM56 of *E. maxima* is a potential immune-boosting antigen at a median dose rate [33]. A lot of experimental studies are present regarding different genes against *Eimeria* in chicken. Moreover, the use of different cytokines is also a part of study during the formulation of DNA vaccines against coccidiosis.

Role of different cytokines in DNA vaccine against Eimeria

Several factors were found which mediate innate immunity in the avian immune system. These immune cells recognize specific pathogen-associated molecular patterns (PAMPS) with the help of their specific pattern recognition receptors. The studies on these immune cells related to coccidiosis and *Eimeria* species emphasized the extraordinary qualities of these cells, results in the discovery of new uncharacterized genes [34]. Different cytokines like IL-1β, IL-2, IL-8, IL-1, IL-15, IL-17, IFN-γ and IFN-α were used in DNA vaccination techniques to boost up immunological properties of vaccines. Some of these cytokines along with their respective references are discussed in Table 1.

Cross protection of DNA vaccines against different Eimeria species

One of the most prominent and eminent advantages of DNA vaccines over ordinary vaccines, observed by different scientists was its cross-protection against other *Eimeria* species. As different species of *Eimeria* have their specific pathogenicity and

Table 2 Cross protection of DNA vaccines against different *Eimeria* species.

Gene	Specie(s) used	Cross protection	Reference
1. pcDNA–TA4–IL-2	*Eimeria tenella*	*Eimeria acervulina*, *Eimeria necatrix* and *Eimeria maxima*	[32]
2. Pvax1-cSZ2-IL-2	*Eimeria tenella*	*Eimeria acervulina*, *Eimeria necatrix*, but not against *E. maxima*	[31]
3. 8 antigens	*Eimeria tenella*, *Eimeria acervulina*, *Eimeria necatrix*, *Eimeria maxima*	*Eimeria tenella*, *Eimeria acervulina*, *Eimeria necatrix* and *Eimeria maxima*	[35]
4. pcDNA4.0 (c)-pEtK2-IL-2	*Eimeria tenella*	*Eimeria acervulina*, *Eimeria necatrix* and *Eimeria maxima*	[39]
5. pJC264–SO7	*Eimeria tenella*	*Eimeria acervulina*, *Eimeria necatrix* and *Eimeria maxima*	[43]

pathogenic sites in the small intestine of poultry. *E. acervulina* infects the upper portion of small intestine while *E. brunetti* infects the lower part of intestine in birds. Similarly, *E. maxima* infects the middle portion, with a small portion of upper and lower intestine. *E. preacox* infects the upper intestine while *E. necatrix* infects the middle portion of the small intestine mostly. *E. mitis* and *E. mivati* infect the upper and middle portion of the intestine while *E. tenella* which is the most important and pathogenic of all infects caeca within the small intestine [42]. A brief diagrammatic view of pathogenic sites of different *Eimeria* species which are mostly responsible for different forms of avian coccidiosis is shown in Fig. 2. The discovery of novel methods and techniques to induce cross-protection against coccidiosis is of valid importance because there are several responsible *Eimeria* species and ordinary vaccines only provide specie specific immunity. Tests on different DNA vaccines formulated until now can induce partial cross-protection against other *Eimeria* species as studied by different scientists. (Table 2).

Dendritic cell-derived exosomes

Dendritic cells (DCs) were found as a good potential source to induce extremely specific acquired as well as innate immunity. DCs makes an immunological pathway through different chemical signals with T-cells and play a fundamental role in the activation of T-cells. DCs are nature adjuvants and are of eminent importance in vaccination techniques. Exosomes separated from DCs having parasitic antigens may become an alternative strategy against coccidiosis. Immature DCs have a low potential to activate T cells [44]. They become mature after phagocytizing any antigen and then migrate towards the lymph nodes where they put antigens in front of T-lymphocytes (Fig. 3). A research-based on a novel strategy to induce immunity against coccidiosis by using DC-derived exosomes [45]. In this study, chicken intestinal DCs were extracted from the antigens of *E. tenella*, *E. maxima* and *E. acervulina* [45]. Isolation of cell-derived exosomes was performed which were later on used as immunogenic agents towards different chicken groups. After challenge with different *Eimeria* species, it was revealed that chickens immunized with DC exosomes showed an increased number of Th2 cytokines, Th1 cytokines, Ag-reactive antibodies and interleukin-16 (IL-16). The results of this study demonstrated that DC-derived exosomes along with incubated AGS could be used as a successful field vaccination against *Eimeria* species. An alternative strategy by using antigen-loaded DCs without free antigens was used against *E. tenella* [46]. The DCs were isolated from the intestines of chickens. Chickens were immunized with Ag-pulsed DCs and challenged with live *E. tenella* infection. After successful completion of the trial, it was proved that immunized chickens showed a higher body weight gain, decrease feed conversion ratio, and reduced oocysts along with a higher anti-coccidial index. This study was the first display of Ag-specific immunity against *Eimeria* by using Ag-loaded DCs.

Use of recombinant proteins as vaccines

Scientists have always explored the ways for the development of new types of vaccines to induce immunity against coccidiosis. The use of recombinant proteins as a vaccine was based on proteins from coccidiosis of bugs in comparison to vaccines taken from live parasites. This vaccine could be used as a mass vaccine for the greater population of chicken flocks to confer immunity [47]. Several examples of recombinant protein vaccines are present, including the contribution of Huang et al. [48], in which a recombinant microneme protein of *E. maxima* (rEmMIC7) was

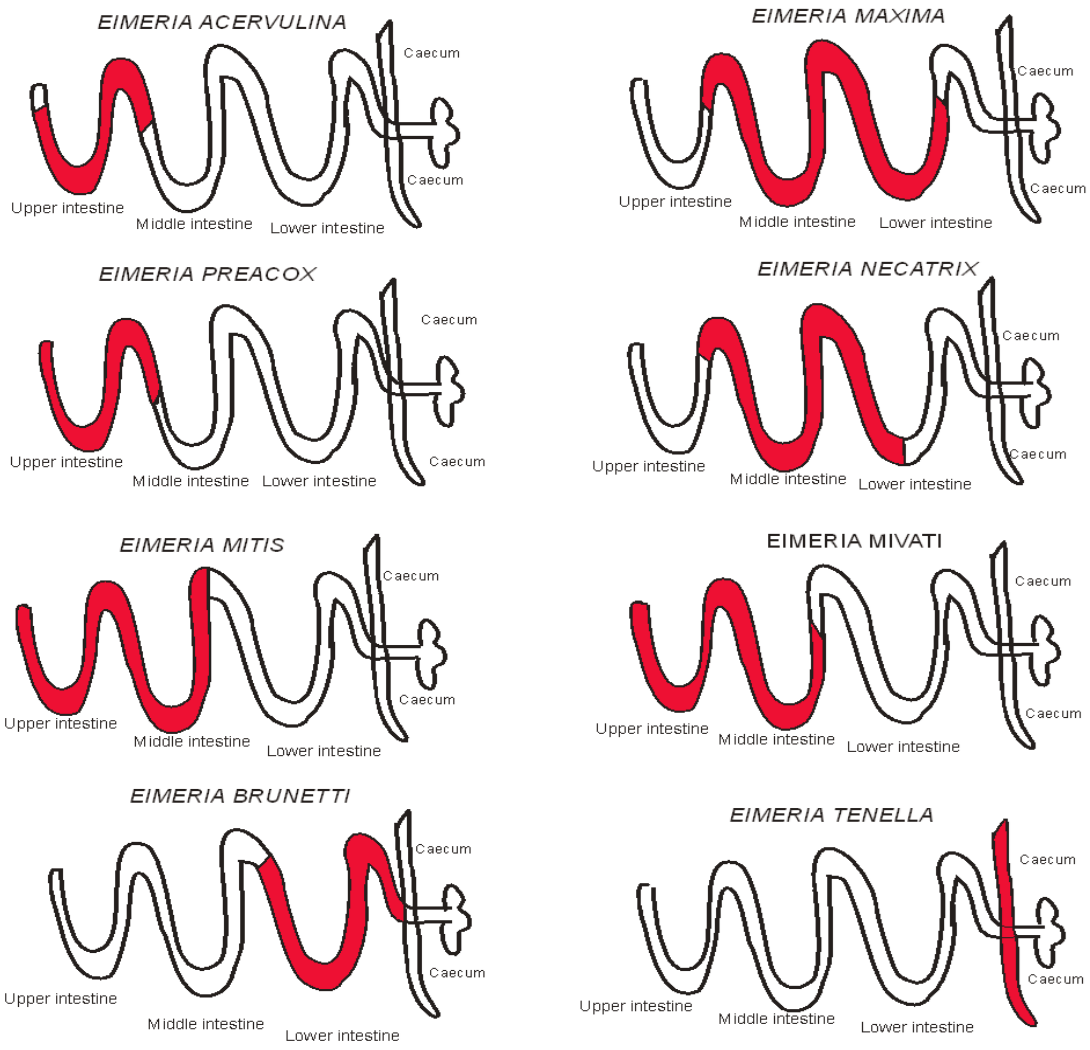

Fig. 2 Specific pathogenic sites for different avian Eimeria species (small intestine).

evaluated along with a DNA vaccine (pVAX1-EmMIC7). The results revealed that both protein vaccine and DNA vaccine elevated antibody titer against *Eimeria* challenge as compared to PBS and pVAX1 control groups. Rabbit antiserum was used against a native protein (F3) of 18 to 27-KD from merozoites of *E. acervulina* (3-1E) [49]. Rabbit antiserum reacted with the recombinant 27-kD recombinant 3-1E protein expressed in Sf9 cells while 20-kD protein was expressed by *E. tenella* and *E. acervulina* merozoites and sporozoites. Monoclonal antibodies were produced against recombinant 3-1E protein by reacting with merozoites and sporozoites of *E. tenella*, *E. maxima* and *E. acervulina*. Lymphocytes of the spleen showed antigen-specific proliferation along with (IFN-gamma) production due to stimulated recombinant 3-1E. This trial proved that cell-mediated immunity can be induced against *Eimeria* species.

In ovo inoculation

The effects of commercial coccidiosis vaccines were determined *in ovo* previously, but there is still a need to check the effects of different types of vaccines *in ovo* accordingly. Inoculation in ovum with the EtMIC2 gene raised anti-EtMIC2 antibody levels at days 10 and 17 following *E. tenella* infection [51]. A highly conserved sporozoite and merozoite of *E. profilin* protein that is a ligand for Toll-like receptor, was an important vaccine candidate since it increased cell-mediated immunity and showed protection to a live coccidial challenge injected *in ovo* [52]. A recombinant (3-1E) protein was used *in ovo* against *Eimeria* in both forms, *i.e.*, alone and along with different cytokines

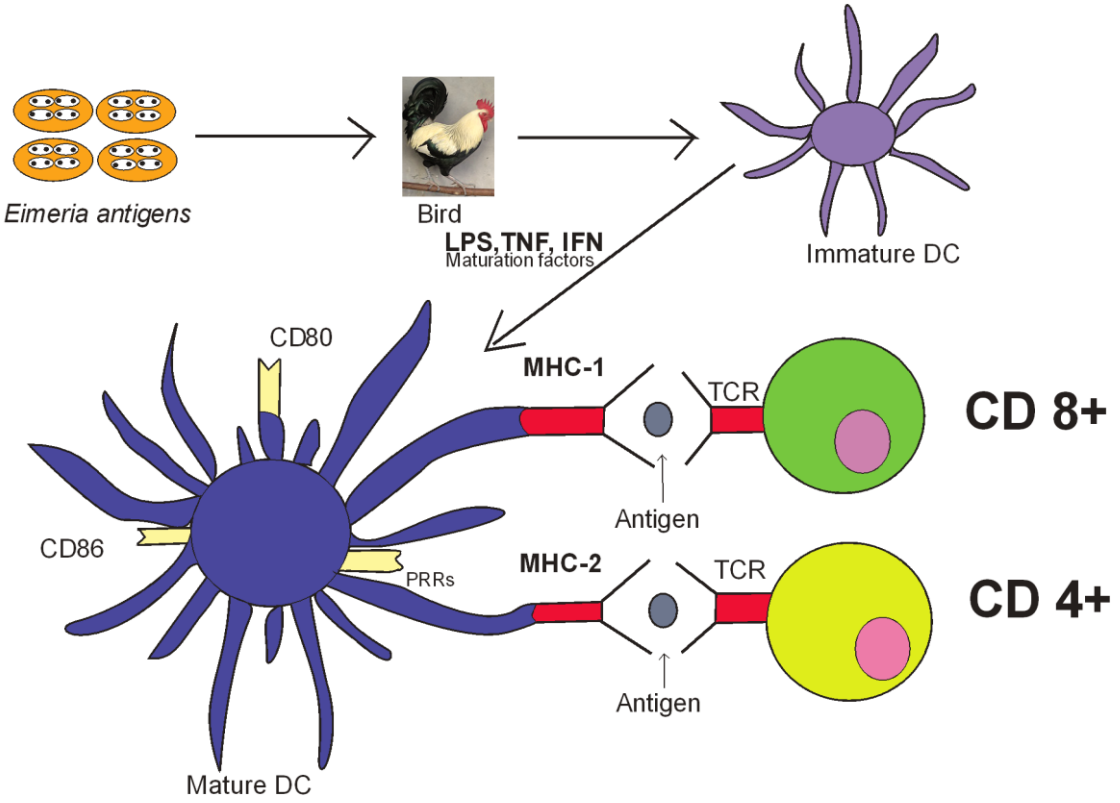

Fig. 3 Maturation of dendritic cells along with activation of CD^{8+} and CD^{4+} T cells in response to pathogens. Adopted from Rehman et al. [50]

like interferon-gamma (IFN-γ), IL-1, IL-2, IL-6, IL-8, IL-15, IL-16, IL-17 and IL-18 [53]. When used alone, vaccine did not show significant results as compared to use with IL-1, IL-16, IL-15 and IL-8. Immunity was evaluated by 14 days post-hatch challenge of *E. acervulina*, which depicts that vaccinated birds showed low oocyst shedding compared to non-vaccinated chickens. *In ovo* inoculation of two recombinant protein vaccines using two cloned *Eimeria* genes, *i.e.*, EtMIC-2 and 3c1E was tested [52]. EtMIC-2 produced high antibody titer alone but along with adjuvant IL-8 and IL-16, the observed effect was much higher. These studies indicated that *in ovo* inoculation of recombinant vaccine may promote immunity against coccidiosis.

Available and experimental vaccines against coccidiosis

There are a lot of vaccines against coccidiosis formulated through different techniques. Some are available commercially while some vaccines are still under experimental progress. Before vaccination, antibiotic therapy was the only solution of coccidiosis but due to lack of prophylactic measures, risks of production losses, mortality and low weight gains were higher. There was a constant need for commercially available vaccines against coccidiosis to confirm prevention. Some available and under-progress novel vaccines for coccidiosis are discussed here.

Live non-attenuated anticoccidial vaccines

Different live non attenuated vaccines were used and still in use against coccidiosis. These vaccines are Coccivac-B, Coccivac-D, Immucox C1, Immucox C2, Inovocox, Inovocox, EM1 and Advent [54].

Live attenuated anticoccidial vaccines

Different live attenuated vaccines were used and still available against coccidiosis. These vaccines include Livacox D, Livacox T, Livacox Q, Paracox, Paracox 5, Nobilis, and COX ATM [55].

Subunit vaccines

CoxAbic is the only subunit vaccine, which has been marketed until now. Subunit vaccine mainly

contains a purified antigen isolated from pathogenic organisms. CoxAbic provokes maternally derived antibodies [56].

Recombinant /DNA vaccines

Several DNA vaccines have been tested in the past few years with marvelous results but still no vaccine is available commercially. Similar is the case with recombinant protein vaccines and dendritic cell-derived exosomes against *Eimeria* species.

Conclusions

Finally, thinking of research from the 1960s until now, it is clear that the role of vaccination to produce immunity against coccidiosis is valid. Before vaccination, a bulk of antibiotics was used against *Eimeria* infections, which were later on crowded with their drawbacks like resistance against a specific antibiotic, etc. Lack of prophylactic measures was also followed by some serious hazards. Antibiotic therapy is always used after the occurrence of disease but till that moment, reduction of production and weight loss along with mortality causes eminent losses. This scenario urged scientists to think of valid prophylactic measures. This all led the formation of vaccination against coccidiosis. Over time, a great progressive change in the vaccination process was made. As coccidiosis is a major disease of poultry, especially in broilers, which affects the economy a lot due to high mortality, weakness of birds and production losses, it is necessary to continue the progress in the vaccination process with great zeal and passion. In this article, it was observed that attenuated and non-attenuated vaccines were mostly used but total protection and eradication of coccidiosis were not noticed. But later studies and novel methods of vaccination showed better results than past. Especially DNA vaccine technology along with cytokines and dendritic cells-derived exosomes are the most favorable vaccines as they not only provoke humoral but also cell-mediated immunity to some extent. Still, there are many questions to be answered in the future, *i.e.*, within the intestine of birds, how *Eimeria* species locate their preferred sites and what are the metabolic pathways these parasites used within and outside the body of the host. While manufacturing novel vaccines, it must be kept in mind that there should be no alternative pathway, which might be used by the parasite to escape itself from immune cells.

Conflict of interest

The authors declare no conflict of interest.

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